REMARKS

Amendments to the Claims

The remarks regarding support for the amendments to claims 8, 48, 55, and 60 and support for new claims 70-75, submitted in the Response of March 17, 2005, still hold.

Claim 10 is now properly cancelled, without strike-through text.

Claims 8, 48, 55, 60, 70, 71 and 73-75 are amended to include the word "cell" between "inner" and "mass-stage", so that step a. now reads: "obtaining a hES cell line from inner cell mass-stage human embryos." This word was inadvertently omitted from these claims, and is needed for reasons of clarity.

With these amendments and new claims, Applicant hereby submits that all pending claims have support in the specification and that no add new matter has been added.

REMARKS SUBMITTED IN RESPONSE OF MARCH 17, 2004 – REITERATED

For continuity and convenience, the remarks regarding prior art that were submitted in the Response of March 17, 2005 are reiterated below:

Claim Rejections under 35. U.S.C. § 103(a) – Obviousness

The Examiner has upheld the obviousness rejections of claims 8, 9, 11, 12, 60 and 65 as being unpatentable over Thomson et al. (1998) *Science* **282**, 1145-1147 in view of Shamblott et al. (1998) *PNAS* (USA) **95**, 13726-13731 and further in view of Yuen et al. (1998) *Blood* **91**, 3202-3209. As the foundation for this rejection, the Office Action states on p. 4 that "Thomson teaches human ES cells that retain the ability to form all three embryonic germ layers," that Shamblott "teaches the production of human EBs from human primordial germ cells" and that Yuen teaches "the production of embryoid bodies in suspension from mouse ES cells."

As discussed in the interview of March 3, 2005, Shamblott et al. teaches production of hEBs from human primordial *germ* cells and Yuen et al. teaches the production of EBs in suspension from *mouse* ES cells, not human ES cells. As clearly

explained by Dr. Benvenisty, "[h]uman EG cells are very different from hES cells" and "any cell line deriving from hEG cells will be dissimilar from cell lines derived from hES cells." See Declaration of September 21, 2004 (Declaration 1), para. 7-8.

Moreover, as stated by Dr. Benvenisty in the present Declaration (Declaration 2) "Thomson never satisfactorily verified that the human ES cell lines that were generated "maintained the potential to form derivatives of all three embryonic germ layers" (see Thomson p. 1146, 1st column, lines 30-32), because he relied on evidence of teratomas generated in SCID mice injected with said cell lines (*id.*, p. 1147, Figure 4) to conclude that his cells actually were pluripotent human ES cells. As pointed out by Shamblott on the first page of the cited reference (see Shamblott, p. 13726, col. 2) "ES and EG cells from some species can form teratocarcinomas when injected into histocompatible or immunologically comprised mice. This property alone may not be a definitive test of stem cell pluripotency, as it has been demonstrated that rat and mouse visceral (yolk sac) endoderm are capable of forming highly differentiated teratomas containing cells of all three embryonic germ layers." Nonetheless, assuming Thomson's cells actually are pluripotent hES cells, if one starts with Thomson's cells, and tries to apply the methods of Shamblott for hEG cells to form hEBs, and then use mouse protocols such as Yuen et al. to direct differentiation, one will not get the results we have seen, not would one in the art even combine these technologies." See Declaration 2, para. 3.

And as first pointed out by Dr. Benvenisty in Declaration 1 and reiterated in Declaration 2, para. 4, "the human EG cells in Shamblott are very different from hES cells, with respect to many features. Any cell line deriving from hEG cells will be dissimilar from cell lines derived from hES cells." In fact, human EG cells are so different from hES cells that Shamblott admits on p. 1372, col. 1, para. 1 of the Results that "Unlike mouse pluripotent stem cells (ES and EG), these human cells were more resistant to disaggregation by trypsin/EDTA-based reagents" and again in col. 2, para. 3 of the Discussion that "The highly compacted nature of these colonies suggests strong cell-cell adhesion. These interactions are *notably* more resistant to trypsin than mouse ES and EG colonies. *Alternative* disaggregation enzymes are currently under investigation" (emphasis added).

As amended, claims 8, 48, 55, 60 and new claims 70, 71 and 73 require the human EBs to be chemically dissociated and then the ES cells are cultured as a monolayer. This is not possible with Shamblott's EG cells. As emphasized by Dr. Benvensity, "More importantly, my methods for forming hEBs and directing differentiation of human embryonic cells derived from those hEBs *require* dissociation of the hES cells initially used, followed by aggregation to hEBs, followed by additional dissociation of the hEBs to obtain dissociated human embryonic cells that can be treated with various factors to direct differentiation." See Declaration, para. 4.

In addition, as stated in the Response and Declaration 1 of September, 2004, Dr. Benvenisty is the first to successfully show how one can direct differentiation in human ES cells derived from human EBs (see also Declaration 2, para. 5). Moreover, the prior art was extremely unpredictable until the Applicant's remarkable breakthrough. At the time the presently claimed invention was filed, efforts by persons skilled in the art to show *in vitro* formation of human EBs had failed, and efforts to form EBs from monkey ES cells (Rhesus and marmoset) had proven impossible or sporadic at best, never mind taking it all a step further to direct differentiation of hES cells derived from human EBs. As pointed out by Dr. Benvenisty "Without the ability to start with actual pluripotent human ES cells, dissociate the hES cells and allow them to aggregate and form true human EBs, and then dissociate the hEBs into dissociated human embryonic cells, one would never be able to look to Yuen et al. and mouse protocols for directing differentiation of the dissociated EB-derived human embryonic cells." See Declaration 2, para. 6

Also, as stressed by Dr. Benvenisty "This second dissociation step is essential for directing differentiation of human embryonic cells derived from hEBs. For these reasons, it was not obvious to produce differentiated cells from human EBs and the establishment of embryoid bodies from human ES cells may be considered a new and not obvious technology since it was developed at a time when all the research reported that such technology was not possible (Reubinoff et al, 2000). As stated in Declaration 1, when I attended a conference in 2001 and presented my results showing human embryoid body formation from human ES cells and subsequent directed differentiation of the human embryonic cells, and many experts in the field simply did not believe me (including

Reubinoff), questioning my results intensely. Therefore, the combination of Thomson, Shamblott and Yuen would never be made by someone on the field trying to form true human EBs and then dissociate them to dissociated human embryonic cells that can be treated with various factors to direct differentiation. And even if such a combination were attempted, it would not be successful." See Declaration 2, para.8.

Therefore, one of ordinary skill in the art would not combine Thomson (hES cells) with Shamblott (hEG cells) and then Yuen (mouse EBs that are further dissociated, cultured and differentiated) because there is no expectation of success, one of the three elements required for a *prima facie* case of obviousness (see MPEP s. 2142). Basically, the combination of Thomson, Shamblott and Yuen would not lead to the presently claimed invention.

Elsewhere in the Office Action the Examiner rejects claims 8-12, 14-16, 48, 51 and 52 as being unpatentable over Thomson et al. in view of Shamblott et al. and further in view of Bain et al. (1995) *Devel. Biol.* **168**, 342-357 (see Office Action, p. 4); rejects claims 8, 11, 13, 48, 51 and 52 as being unpatentable over Thomson et al. in view of Shamblott et al. and further in view of Bain et al. and Wobus et al. (1988) *Biomed. Biochim. Acta* 47, 965-973 (see Office Action, p. 5); and rejects claim 55 as being unpatentable over Thomson et al. in view of Shamblott et al. and further in view of Bain et al. and Wobus et al. (see Office Action, p. 7).

The failings of Thomson et al. in combination with Shamblott et al. and Yuen et al. have been described above. Relative to the addition of Bain et al., and Wobus et al. without the primary combination of Thomson, Shamblott and Yuen, persons skilled in the art would not additionally combine Bain and/or Wobus. Moreover, Dr. Benvenisty stated previously in his Declaration of September 21, 2004 (Declaration 1), that "Bain reports the treatment of mouse ES cell-derived embryoid bodies with retinoic acid (RA) and their differentiation into the neuronal lineage. The technology of developing embryoid bodies from mouse ES cells and directing their differentiation was known and established at the time of the present invention. However, the techniques and protocols that applied for mouse ES cells were not adequate for human ES cells. Therefore, the establishment of embryoid bodies from human ES cells may be considered a new and not obvious

technology, since it was developed at a time when all the research reported that such technology was not possible (Reubinoff et al, 2000)." See Declaration1, para. 18.

Similar considerations argue against additionally combining Wobus to the Thomson, Shamblott, Yuen combination (for a more detailed discussion of Wobus, see Declaration 1, para. 22, submitted September 21, 2004).

For all the foregoing reasons, Applicant respectfully submits that the pending claims are not obvious in light of the cited prior art. Reconsideration of the claims and withdrawal of the obviousness rejections are therefore requested.

CONCLUSION

Claims 1 – 7, 17 – 47, and 10 are cancelled, but Applicant reserves the right to pursue such claims in a later related application. Claims 49, 50, 53, 54, 56-59, 61-64 and 66-69 are withdrawn without prejudice, but Applicant requests consideration for allowance if the base claims upon which these withdrawn claims depend (i.e. claims 8, 48, 55 and 60) are found allowable.

In view of the arguments and amendments presented, Applicant respectfully submits that all pending claims are now in condition for allowance. Reconsideration of the claims and a notice of allowance are therefore respectfully requested.

Applicant believes that no extension of time is required. However, in the event that an extension is required, however, this conditional petition for an extension of time is requested. If any fees are required for the timely consideration of this application, please charge deposit account number 19-4972.

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